ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT DEFENSE IN ALZHEIMER'S DISEASE

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Abstract- The etiopathogenesis of Alzheimer's disease (AD) is still unclear. However, long-term oxidative stress is believed to be one of the major contributing factors in progression of neuronal degeneration and decline of cognitive function in AD. In order to assess the presence of oxidative stress in AD, we examined the enzymatic activities of the erythrocyte Cu-Zn superoxide dismutase (Cu-Zn SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and plasma level of total antioxidant status (TAS) in AD and control groups (age and sex-matched). The results showed that the Cu-Zn SOD activity was significantly higher and the level of GSH-Px and TAS activities were significantly lower in AD subjects compare to that in the control group (2111 ± 324 U/grHb, 43.7 ± 11.6 U/grHb, and 1.17 ± 0.23 mmol/l compared with 1371 ± 211 U/gr Hb; t = -2.17, P = 0.036, 56.3 ± 9.5 U/gr Hb; t = 3.8, P = 0.014, and 1.54 ± 0.2 mmol/l; t =11.18, $P < 0.001$, respectively). While, the erythrocyte CAT activity was lower in AD subjects compared to the control group, the difference was not statistically significant (t = 1.3, P = 0.15). These findings support the idea that the oxidative stress plays an important role in the pathogenesis underlying AD neurodegeneration. In addition, the enzymatic activity of the erythrocyte Cu-Zn SOD and GSH-Px and the plasma level of TAS can be used as a measure of the oxidative stress and a marker for pathological changes in the brain of patients with AD.

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Key words: Alzheimer's disease, Oxidative stress, Superoxide dismutase, Glutathione peroxidase, Catalase, Hydrogen peroxide

INTRODUCTION

The etiopathogenesis of Alzheimer's disease (AD) is still unclear (1-3). Recent findings indicate that cellular events involving oxidative stress may be a basic mechanism of neurodegenerative disease.

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Severe oxidative stress progressively leads to cell dysfunction and ultimately cell death. Oxidative stress is an imbalance between pro-oxidants and/or free radicals on one hand, and anti-oxidizing systems on the other (2-7). Oxidative stress results from generation of oxygen free radicals, hydrogen peroxide, hydroxyl radical, hydroperoxide, dioxygen and nitric oxide, collectively termed as reactive oxygen species (ROS). ROS is hypothesized to be main etiologic factor for progressive and specific neuronal degeneration which is observed in the AD

(1, 8, 9). These ROS are highly reactive toward protein, lipids and DNA molecules causing damage to these macromolecules and possibly leading to dysfunction or death of the cell (8, 10).

There are many intrinsic free radical scavenger systems, which involve enzymatic and no enzymatic reactions. One of the enzymatic antioxidant defense systems is copper-zinc super oxide dismutase (Cu-Zn SOD) that converts super oxide radicals to hydrogen peroxide (H_2O_2). Glutathione peroxidase (GSH-Px) and catalase (CAT) will then convert H_2O_2 to a water molecule. Cu-Zn SOD, GSH-Px, and CAT together provide the primary antioxidant defense mechanism (4, 8, 11, 12).

The non-enzymatic antioxidant defense system includes ascorbic acid (vitamin C), α- tocopherol (vitamin E), glutathione (GSH), β-Carotene, and vitamin A. There is a balance between both the activities and intracellular levels of these antioxidants that are essential for the survival of organisms and their health (4, 10, 13-15). It is known that the brain bears relatively low antioxidant protection, and also contains high levels of polyunsaturated fatty acids that makes it prone to increased lipid peroxidation (3, 9, 16).

Recent studies indicate that amyloid beta- peptide (Aβ) can be neurotoxin by mechanisms involving the generation of H_2O_2, ROS and lipid peroxidation (5, 17-20).

It has been suggested that the brain of the AD patients are affected by inordinate oxidative stress (21). This may be due to an increased SOD and/or decreased GSH-Px and CAT activities, leading to elevation of H_2O_2 concentration in AD. The generated H_2O_2 is used for hydroxyl radical production via Fenton and Haber-Weiss reactions (4, 6, 8, 12, 22). Thus, identifying marker(s) that can be used as a measure of the oxidative stress associated with the pathologic changes in the brain of patients with AD is highly beneficial.

In this case-control study we investigated the level of the enzymatic activities of erythrocyte Cu-Zn SOD, GSH-Px, and CAT, factors involved in an antioxidant defense mechanism, and the total antioxidant status (TAS) of plasma in AD patients.

MATERIALS AND METHODS

Our case-control study consisted of 91 AD cases (40 males and 51 females with mean age of 75 ±10 years) with no reported family history of AD or dementia and 91 unrelated controls (40 males and 51 females with a mean age of 73.5 ± 11.5 years). AD cases were recruited from the Shariati Hospital and Roozbeh neurology and psychiatry center at University of Tehran, in Tehran, Iran. The diagnosis of probable AD was based upon the NINCDS/ADRDA clinical diagnostic criteria (23), brain CT-scan and/or MRI. Subjects were excluded if they had a family history of dementia (more than one first-degree relative with any type of dementia). Volunteer control subjects were consisted of age/sex and ethnic background matched cases, who were spouses of clinically ascertained AD or dementia patients. These subjects were chosen based on their medical history and physical examination. Their cognitive function was assessed using Mini Mental State Examination (MMSE) (24) and they did not show signs of dementia.

A written and informed consent was obtained from the subject or his/her legal guardian and twelve hours after fasting, blood samples were collected from each subject. The bloods were centrifuged at 500g for 15 min and serums were collected. To obtain packed erythrocytes, the remaining erythrocytes were washed repeatedly with an isotonic solution of NaCl (0.9%) until a colorless supernatant was observed. To obtain erythrocyte hemolysate, 500 µl packed erythrocyte was hemolyzed by addition of four volumes of cold double distilled water. The resulting suspension was centrifuged twice to eliminate all of the cell membranes as described (25). The hemolysates were used to determine Cu-Zn SOD, GSH-Px, and CAT activity.

Erythrocyte Cu-Zn SOD and GSH-Px activities were measured using the commercially available kits (Randox. Lab. Ltd. Ireland cat No.SD125 and RS 505 respectively). Erythrocyte CAT activity was measured by the method of Aebi *et al.* (22). Plasma TAS level was measured using commercially available kit (Randox, Lab. Ltd. Ireland cat No. NX 2332).

Data were expressed as mean ± standard deviation (SD). Two-tailed Student's *t* test were used to compare the data between AD and control groups. Statistical significance was assumed at the $P < 0.05$ levels. The SPSS statistical software package version 11.5 was used for all of the statistical analysis.

RESULTS

The age, gender, erythrocytes Cu-Zn SOD, GSH-Px, and CAT activities and plasma TAS concentration in AD and control groups are given in Table 1. The average age of AD subjects (75 ± 9.3 years) was slightly higher than that of the control (73.2 ± 11.8), but gender distribution was the same in both groups. The mean plasma concentration of TAS and the activity of erythrocyte GSH-Px were lower in those with AD than in control subjects (1.17 ± 3.5 mmol/l vs 1.54 mmol/l; t=11.18, $P < 0.001$ and 43.7 ± 11.6 U/gr Hb vs 56.3 ± 9.5 U/gr Hb; t=3.8, $P = 0.014$, respectively). As shown in Table 1, the erythrocyte CAT activity was also lower in AD subjects compared to control, the difference, however, was not statistically significant (t=1.3, $P = 0.15$). The activity of erythrocyte SOD, on the other hand, was significantly higher in those with Alzheimer's disease than the control subjects (2111 ± 324 U/gr Hb vs 1371±211 U/gr Hb; $t = -2.17$, $P = 0.036$).

Table 1. Oxidative stress parameters in AD patients and normal controls*

Parameters	AD	Control	*t*†	*P*†
Age (year)	75 ± 9.3	73.2 ± 11.8	-1.15	0.26
Sex (M/F)	40/51	40/51	--	--
SOD (U/gr Hb)	2111±324	1371±211	-2.17	0.03
GSH-Px (U/gr Hb)	43.7±11.6	56.3±9.5	3.8	<0.01
CAT (U/gr Hb)	117±15	127±12	1.3	0.15
TAS (mmol/L)	1.17±0.23	1.54±0.2	11.18	<0.001

Abbreviations: AD, Alzheimer's disease; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; TAS, total antioxidant statues.
* Data are given as mean ± SD.
†By Student's *t* test.

DISCUSSION

Oxidative stress is believed to play an important role in neuronal dysfunction and ultimately cell death (12). AD has been hypothesized to be associated with oxidative stress (4, 7). In this study we have found that the activity of erythrocyte Cu-Zn SOD is substantially increased in AD patients compared with age-matched control subjects, but erythrocyte GSH-Px activity and plasma TAS concentrations are reduced. These observations suggest a role for oxidative stress in the pathogenesis of AD neuro-degeneration (1, 4, 16). The high level of Cu-Zn SOD and the low level of GSH-Px and CAT enzymes in AD patients may result in accumulation of H_2O_2 and excessive free radicals in their brain, leading to neurodegeneration. This is consistent with pervious studies demonstrating a decreased GSH-Px (4, 12, 20, 26) and an increased SOD (2, 4-6, 12, 27-29) activity in Alzheimer disease and advanced aging compared to healthy controls. A significant increase in Cu-Zn SOD activity has also been found in the brain of AD patients (11) along with a defect in the metabolism of ROS that causes neuronal apoptosis (30). It has been suggested that the systems that detoxify reactive oxygen species, including enzymatic antioxidants such as glutathione peroxidase (GSHPX) and catalase are decreased in the hippocampus of AD, while superoxide dismutase (SOD) is increased (31). Ceballos *et al.* (32) however, reported that activity of SOD and GSH-Px are similar in AD and the same age control groups. While Licastro *et al.* (27) reported that the activity of GAH-Px was increased in AD patients and Marcus et al (33) reported activity of SOD was decreased in AD patients.

As shown in table 1, we have also found a significant decrease in the TAS concentration in the AD patients compared to the control. Antioxidant activity decreased in the presence of low level of TAS leading to a compensatory elevation of SOD activity.

The decrease of antioxidant activity in the presence of low level of TAS leading to a compensatory elevation of SOD activity. Hyper production of H2O2 and inadequate antioxidant enzyme activities resulted in cell damage. Also, high

free radical production in AD patients leads to a rapid consumption of plasma antioxidants that is not concomitant with activation of antioxidant enzymes (4).

Our finding is in accordance with previous studies and the epidemiological data, demonstrating that antioxidants may have a beneficial effect on many age-related diseases, such as AD (16, 34). Furthermore, even though studies support the hypothesis that subjects with AD are malnourished-particularly in the last phase of the disease—more recent by several researches have demonstrated lower plasma antioxidant levels in the early AD stages in well-nourished subjects (12). The plasma non-enzymatic antioxidant profile of AD patients shows that the major components of the antioxidant defense system are affected in this condition. In fact, recently an epidemiological prospective cohort study shows that high dietary intake of Vitamin C and Vitamin E may lower the risk of Alzheimer's disease (11).

Finally, the measurement of peripheral markers of oxidative stress (3, 4, 33, 34) could be used in order to biologically assess the efficacy of antioxidant supplementation in AD and mild cognitive impairment (MCI). Recent studies indicate that amyloid beta- peptide (Aβ) can be neurotoxin by mechanisms involving the generation of H_2O_2, ROS and lipid peroxidation (5, 17-20). β amyloid toxicity is eliminated by free radical scavengers. The ability of SOD to protect against Aβ cytotoxicity suggests that the activation of the pathway for generation of ROS occurs upstream of superoxide (SOD activity increased). The antioxidant enzymes CAT and GSH-Px are also associated with senile plaques (30). The ability of CAT and GSH-Px to breakdown H2O2 generated in response to Aβ is a suggestive mechanism for CAT and GSH-Px protection (35).

Some in vitro studies have observed that b-amyloid aggregated peptide, a characteristic feature of AD, is toxic to neurons in culture likely through generation of free radicals and by induction of lipid oxidation (36). Marcus et al (33) have reported that the temporal lobes of AD patients consistently showed significant differences in activity for the above studied three enzymes, suggesting that abnormalities in the antioxidant system may lead to neuronal cell death in AD. These results correlate well with the regional pathology of AD and provide additional evidence for a relationship between the development of AD and a breakdown of the antioxidant system. A causal relationship between these results and the development of AD remains to be determined. If causal, then an alteration in the gene for SOD, CAT, GSH-Px, or three may have occurred. If not causal, then the abnormalities in the antioxidant system, observed in these studies, may be due to the effects of AD. Our studies indicate that learning about the antioxidant defense mechanism in AD may lead to unraveling the role of free radicals in this neuro-degenerative disease. In addition, our data suggests that the erythrocyte and the plasma level of Cu-Zn SOD, GSH-Px, and TAS can be used as a marker for pathological changes in the brain of patients with AD.

Conflict of interests

The authors declare that they have no competing interests.

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